AN INTRODUCTION TO IMMUNE SYSTEM WITH SPECIAL FOCUS ON VACCINES FOR SARS-COV-2: PART II

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This is the second part of the two-part article. It will deal with vaccines, vaccine design and types of vaccines, trials during the development of a vaccine, and the process of approval by concerned authority for public use. It will also discuss on the various strategies used in vaccines against SARS-CoV-2 that cause COVID-19 by taking some examples of the recently approved vaccines

Keywords: Immune system, vaccines, vaccine trials, vaccine design.

Introduction

In part I of the two-part article, we have discussed about pathogens and how the immune system responds to the pathogens. We have also discussed about the types of immune system — innate immune system and adaptive immune system. Under the latter, we discussed about humoral immunity and cell-mediated immunity. The sequence of events that follow SARS-CoV-2 infection have also been dealt in brief. This article, which is the second part of the two-part article, will focus on vaccines and vaccine design, how vaccines are developed and how they provide immunity to specific diseases especially in the context of the coronavirus, SARS-CoV-2. The article will also discuss on the various steps involved in the process of vaccine trials, approval and dissemination. Readers will also get familiarized with the few approved vaccines for COVID-19.

Vaccines

Innovative therapeutic interventions have been in use for centuries and are critical to prevent and treat human and animal diseases. It could be injecting antibodies, those molecules that would subdue the virus, either made synthetically in the lab or from the blood of people who have recovered (Plasma Therapy). Sometimes drugs could help by entering our cells and blocking the virus from copying itself and making the virus less deadly. But the best way to beat an infection and stop a pandemic is using a vaccine. And it does so by taking advantage of something that our bodies have evolved over millions of years — Memory. As discussed in Part I, adaptive immune response produces memory B and T cells which will mobilize rapid and robust response upon re-exposure of the same antigen in case of actual infection. While drugs are administered to treat a medical

problem, vaccines are administered to prevent the appearance of a medical problem. Some exceptions can be found in both classes, such as cancer vaccines (vaccines that are administered after detection of the problem) and proton pump inhibitors (drugs that are often administered to prevent gastric problems in co-therapy with other drugs). Notwithstanding, vaccines and drugs are similarly regulated both in research and development, manufacturing, clinical trials, government approvals, and regulation. In the big picture, a vaccine is a special type of drug. There are also few other differences. For example, for vaccines, dose, time, route and frequency are generally established. In contrast, since drugs are used for patients with different conditions, dose, time and frequency of drug administration are often very difficult to determine (He et al., 2012). Also, unlike most drugs, whose benefit is restricted to the individual who takes the drug, vaccines have the potential for far-reaching effects that encompass health service utilization, general health and well-being, and ultimately economic productivity. The impact of vaccines is measured by evaluating effects directly on the vaccinated individuals, indirectly on the unvaccinated community, the epidemiology of the pathogen (such as changing circulating serotypes) and additional benefits arising from improved health. Aside from the protection of the individual, the broader success of vaccination is dependent on achieving a level of coverage sufficient to interrupt transmission of the pathogen (Doherty et al., 2016). This suggests that vaccination of a huge chunk of a population is necessary to interrupt transmission of the pathogen through herd (population) immunity or herd protection (i.e., the indirect protection provided to unvaccinated individuals in a population resulting from most of the population having got vaccinated).

Early attempts in the development of vaccines were based on the observation that people surviving a disease, for example, a person who has recovered from Small Pox, was protected against further occurrence of that particular disease. From the vantage point of today's understanding, we know that it is the immune response and its memory (be it B or T cells). The goal of the vaccination is to fake an infection that exposes the body to an antigen that will not cause disease but will provoke an immune response that can block or kill the virus. To fake this infection, there are various ways by which viral antigens or viral toxins or viral proteins can be delivered as vaccines which are discussed in the next section.

Vaccine design, strategies, advantages and disadvantages

Vaccine design has made significant strides in the last century, evolving from serendipity to a more rational approach thanks to our understanding of immunological mechanisms, our insight into the molecular details of virus replication and pathogenesis (Delany et al., 2014).

Vaccines are broadly divided into two — live, attenuated (weakened in a laboratory) vaccines and non-live/ killed/ inactivated (which have lost its pathogenicity but not its antigenicity) vaccines. Two essential things that have to be taken into account while vaccine development is first safety, considering that vaccine is given mostly to healthy individuals, and secondly efficiency or efficacy, to overcome an infection intended for (Payne, 2017). A key consideration

in developing a vaccine is whether or not the vaccine contains a source of live infectious material. Vaccines containing live infectious agents carry a virus (or bacteria) that replicates just enough to stimulate a protective response in the vaccine recipient but not enough to cause harm. However, since they contain live infectious agents, they may revert to its virulent form and cause the disease, but this happens very rarely. Vaccines that are not live and that do not contain infectious agents, called Non-Live Vaccines, do not contain sources of infectious material and therefore do not cause disease. Hence they generally have a good safety profile, even in immunocompromised individuals. However, a drawback of these vaccines is that immunogenicity and duration of protection tend to be less compared to live vaccines, and they may require several doses to improve immunogenicity. Many times adjuvants are added to the vaccine. Adjuvants are substances that enhance and modulate the immunogenicity of the antigen. Adjuvants are usually not needed for live attenuated vaccines because the viruses in

Table 1
Overview of the advantages and disadvantages of the major vaccine strategies and examples of licensed vaccines

<table>
<tr><td rowspan="3"></td><td>Vaccines that contain live or infectious Particles (Live Attenuated Vaccines)</td><td colspan="5">Vaccines that do not contain Live or infectious Particles: Non-Live Vaccines (Inactivated Vaccines)</td></tr>
<tr><td rowspan="2">Attenuated or Weakened Live Pathogen Vaccine</td><td rowspan="2">Inactivated or Killed Pathogen Vaccine</td><td colspan="4">Subunit Vaccines</td><td rowspan="2">Viral Vector Vaccine</td></tr>
<tr><td>Protein Vaccine</td><td>Toxoid Vaccine</td><td>Virus-like Particles Vaccine</td><td>Nucleic Acid Vaccine</td></tr>
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Strategy employed	It is obtained by repeated passing of the virus through a series of in-vitro cell cultures. With each passage, the selected virus progressively loses its ability to infect and replicate in the human host but causes a minimal infection.	It is produced by inactivating preparations of whole pathogens by heat, radiation, or chemicals such as formalin or formaldehyde. Inactivation destroys the pathogens' ability to replicate and cause the disease but maintains its immunogenicity	Here antigenic proteins are purified(Extracted) from preparations of the whole pathogen or produced by recombinant genetic engineering.	Some bacteria such as Clostridium tetani, Clostridium difficile cause disease by releasing pathogenic toxins. These vaccines for such diseases are produced by detoxifying the toxin using heat, chemicals but retain the antigenic potency (e.g., formaldehyde), or both.	Based on the observation that the expression of certain viral proteins leads to the spontaneous assembly of particles structurally similar to the original viruses	Nucleic acid vaccines encode for pathogen antigen. These vaccines work by inserting DNA or RNA into vectors(Delivery vehicles) which carry and release the same into host cells. The administered mRNA or DNA uses the host cell transcription and translation machinery to produce viral antigen.	It is a recombinant vaccine which uses a vector to carry the recombinant genetic material but it cannot replicate. For e.g., the chimpanzee adenovirus encodes the SARS-CoV-2 Spike protein (i.e., the Spike protein gene of SARS-CoV-2 is added in the DNA of the adenovirus). Thus the adenovirus can produce the Spike protein but cannot replicate.

Advantages	Usually, produce robust Cell-mediated and Humoral immune responses with just one dose. Long-lasting immune response (sometimes lifelong).	Safe as the pathogen is dead. Transport and storage.	Safety during production. It can be safely administered to immunocompromised individuals. No infectious agent is required while handling.	Non-infectious. They are used as carrier proteins due to good immunogenicity	Combine the efficacy of attenuated vaccines and safety of subunit vaccines. Scalability of production. Their size is ideal for the uptake of the cell.	Scalability. Fast design and development. Extremely safe. No infectious agent handling is required. Can induce humoral and cellular responses.	Admistration of this vaccine will not cause COVID-19. There is drastic reduction in the rate of infection or severe infection after two dose of this vaccine.
Disadvantages	Safety issues in immunocompromised individuals. Development of the vaccine takes time. Though very rare, but potential to revert back to a form able to cause the disease (e.g. Oral polio vaccine).	Large quantities of the pathogen need to be processed. The inactivation process can affect antigen immunogenicity. Antibody titers reduce over time. Need several booster doses.	Low immunogenicity. Need several booster doses and adjuvant. Production is limited by antigen production scalability.	Vaccines target only the toxin and do not prevent infection by the pathogen. No herd protection. Priming and boosting necessary.	The assembly of particles is sometimes challenging.	DNA vaccines require a unique delivery platform. mRNA vaccines exhibit instability and require storage at -20°C	Storage and transportation is sometimes an issue as they require very low temperature. For example, Pfizer COVID-19 vaccine needs to be stored below minus 40°C

Licensed Vaccines that use this strategy	Mumps, Measles, rotavirus, Rubella, varicella, Oral Polio, Yellow Fever, Chickenpox, BCG	Whole-cell pertussis, rabies, Polio, Hep A, Covaxin and CoronaVac for COVID-19	Hep B, Hep C, Influenza, Acellular pertusis, HPV	Tetanus, diphtheria, acellular pertussis	HPV, Hep B	Moderna and Pfizer/ BioNTech COVID-19 vaccine	Oxford–Astra-Zeneca COVID-19 vaccine (Eg. Covishield, Vaxzevria), Hepatitis B

the vaccine actively replicate and self enhance the immune response. However, they are frequently used for non-live vaccines because they contain fewer antigens and lack some of the intrinsic components present in the whole pathogens that trigger the innate immune response so that an effective downstream adaptive response is less likely to be achieved (Vetter et al., 2018). For almost a century, aluminum salts (also known as Alum) were the only adjuvant approved worldwide, and they are still in use. Table 1 discusses the strategy, advantages, and disadvantages of various vaccines (CDC, 2015; Kyriakidis et al., 2021; Vetter et al., 2018).

How trials are done, volunteers chosen, phases of trials, and time it takes for mass use

Each vaccine at the time of development must first undergo screenings and evaluations to determine which antigen should be used to invoke an immune response. This preclinical phase involves an experimental vaccine that is first tested in animals to evaluate its safety and potential to prevent disease.

If the vaccine triggers an immune response, it is then tested in humans in three phases.

Phase 1

This phase involves giving the vaccine to a small number of volunteers to assess its safety, to confirm that it generates an immune response, and determine the lower and upper limit of dosage. Generally, in this phase, vaccines are tested in young, healthy adult volunteers.

Phase 2

The vaccine is then given to several hundred volunteers to further assess its safety and ability to generate an immune response. Participants in this phase have the same characteristics (such as age, sex) as the people for whom the vaccine is intended. This phase involves multiple trials to evaluate various age groups and different formulations of the vaccine. A group that did not get the vaccine is usually included in the phase as a comparator group (Placebo group; many a time innocuous saline water is injected) to determine whether the immunity achieved in the vaccinated group is attributed to the vaccine or has happened by chance.

Phase 3

In this phase, the vaccine is given to thousands of volunteers – and compared to those who receive a comparator product – to

determine if the vaccine is effective against the disease it is designed to protect against and study its safety in a much larger group of people. Most of the time, phase three trials are conducted across cities, within a country and foreign to assure the vaccine performance findings apply to many different populations.

During phase two and phase three trials, the volunteers and the scientists conducting the study are shielded from knowing which volunteers had received the vaccine being tested or the comparator (placebo) product. This is called "blinding," which is necessary to assure that neither the volunteers nor the scientists are prejudiced in their assessment of safety or effectiveness by knowing who got which product (WHO, 2020).

Role of Government agencies and other public organizations

When the results of all these clinical trials are available, a series of steps are taken, which includes reviews of efficacy and safety for regulatory and public health policy approvals. Officials then closely review the report and decide whether to authorize the vaccine for use. A vaccine must show clear evidence that it is safe and effective across a broad population before being approved and introduced into a national immunisation program. The bar for vaccine safety and efficacy is exceptionally high, considering that vaccines are given to people who are healthy and specifically free from the illness. Further monitoring takes place even after the vaccine is introduced, enabling scientists to keep track of vaccine impact and safety even as they are used in many people over a long period. The data obtained is used to adjust the policies for vaccine use and optimize their impact. Once a vaccine is in use, it must be continuously monitored to ensure it continues to be safe (WHO, 2020). Governments are also involved in something called Vaccine diplomacy. The COVID-19 vaccine — one of the worlds' most in-demand commodities today — has become a new currency for international diplomacy. Countries with the means and the know-how are using vaccines to find favor or thaw frosty relations. As such, India, the unmatched vaccine manufacturing powerhouse, is doling millions of doses to friendly neighbors and needy countries.

SARS-CoV-2 Vaccine strategies: A review of few approved vaccines

COVID-19 is caused by new positive-strand RNA coronavirus (SARS-CoV-2), which belongs to the Coronaviridae family, along with the severe acute respiratory syndrome (SARS) and the middle east respiratory syndrome (MERS) coronavirus. The majority of vaccines for COVID-19 employ administration of viral antigens or viral gene sequences aiming to induce neutralising antibodies against spike protein (Kyriakidis et al., 20210. Herein, we discuss the different strategies that were used for vaccine development against SARS-CoV-2.

CoronaVac, developed by Sinovac Biotech in China, is a purified inactivated virus alum-adjuvant vaccine. It is formalin-inactivated (Ref. 1). The vaccine is administered in two doses, 14–28 days apart. The phase-III trials included 8870 participants from Brazil, Indonesia, and Turkey (Kyriakidis et al., 2021). Reports have shown that it was 50.4 per cent effective at preventing severe and mild COVID-19 in late-stage trials (Ref. 10).

Vaccines based on inactivated pathogens are produced by inactivating preparations of whole pathogens by heat, radiation or chemicals such as formalin or formaldehyde. Inactivation destroys the pathogens' ability to replicate and cause the disease but maintains its immunogenicity so that the immune system can still recognize the targeted pathogen. Inactivated vaccines present some technical challenges as a disadvantage. The inactivation process can sometimes damage the antigens leading to suboptimal immunogenicity. Therefore these need several boost doses to produce strong immune responses. Besides these vaccines need the addition of adjuvants. While response induced is less than Live attenuated vaccines, these vaccines are easily handled, generally less expensive, and much safer (Forni et al., 2021).

Covaxin, developed by Bharat Biotech and the Indian Council of Medical Research, is another inactivated vaccine rolled out for use against COVID-19. The vaccine was developed by inactivating an Indian strain of the novel coronavirus isolated by the Indian National Institute of Virology. A Phase 3 trial of 26,000 participants is still underway as this article was being written. However, it has been approved, based on the positive results shown in phase I/II by the Drugs Controller General of India (DCGI), which is responsible for approval of licenses of specified categories of drugs such as blood and blood products, IV fluids, vaccines, and sera in India. Covaxin is a two-dose 6µg vaccine administered 28 days apart (Ref. 10).

Moderna and Pfizer/BioNTech have developed vaccines based on mRNA techniques. This is the first mRNA vaccine licensed to be used in humans. How mRNA and DNA vaccines induce immunity is provided in figure 1. Researchers started with the genetic sequence of the COVID-19 and worked backward to find the mRNA that expresses the spike protein. This mRNA of the SARS-CoV-2 is incorporated in the lipid nanoparticle. Upon vaccination, lipid nanoparticle fuses with the host cell membrane. Here nanoparticle releases mRNA. Translation of mRNA to proteins takes place without tampering with the nucleus of the host cell (i.e., in the cells of human body). These proteins are then presented on the cell by MHCs. Here's when adaptive immune response kicks in. Helper T cell recognizes and releases cytokines that signal B cells to proliferate and produce antibodies, and the memory of the same is stored if an actual infection does occur [Kyriakidis et al., 2021].

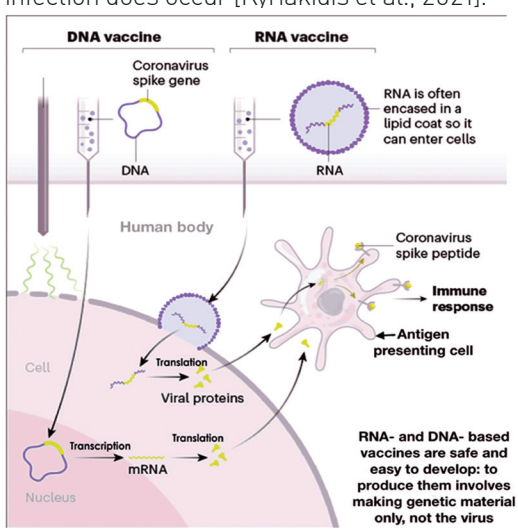

Fig. 1. Inducing immunity by Nucleic and Vaccines: Spearhead Candidates for COVID-19. [Ewen Calaway, 2021]

While Moderna had 30000 participants in phase III and reported 94.1 per cent efficiency, Pfizer had 44000 participants and achieved

95 per cent efficiency overall and 94 per cent in the age group of 65 years and above (Ref. 1).

Covishield, which is rolled out by Serum Institute of India, the largest Vaccine manufacturer in the world capable of producing 5000 doses per minute, is based on the vaccine developed by Oxford University and AstraZeneca. In this case, a DNA molecule derived from the S-protein of the SARS-CoV-2 virus is inserted into Chimpanzee Adenovirus. When this virus (not pathogenic to the human body) is injected, it fuses with the host cell and releases the DNA inside. DNA gets converted to mRNA by transcription. mRNA gets converted to viral proteins through translation which is later presented for adaptive immune response by MHCs (Kyriakidis et al., 2021). Covishield had 30000 participants with an efficiency of 62.14 per cent overall and about 90 per cent in the age group of 18–55 years. Covishield is administered in two doses between 12–16 weeks apart. Janssen Vaccines, a company owned by Johnson & Johnson, has developed JNJ-78436735 (formerly known as Ad26.COV2.S), a single-dose COVID-19 vaccine using the same DNA technique. They had whopping 90000 participants in phase III trials and were 66 per cent effective overall (Ref. 10). These two nucleic acid techniques have saved us months of time and tons of money. Other traditional vaccine development strategies, such as attenuated virus vaccines, although historically leading to very successful vaccines against viral diseases, require long cell culturing processes to achieve attenuated strains. In these two cases, the only ingredient required is the genetic sequence of the SARS-CoV-2 virus [Kyriakidis et al., 2021].

Conclusion

Traditionally, it takes 10 to 15 years to develop a vaccine, although there are exceptions. It took just four years to develop and secure approval for a mumps vaccine that was first licensed in 1948 and 5 years in the case of the Ebola vaccine, which was approved in December 2019. But, what has happened with COVID-19 vaccine development is astonishing with the research work being carried out at a breathtaking pace and rolling of vaccines for use in less than a year. The latest technologies, innovative platforms, and state-of-art labs and manufacturing units, along with overlapping and truncating few regulatory procedures without compromising safety, have helped us blaze through so quickly. For a world to be free of the current pandemic, WHO advocates that at least 60 per cent of the human population be vaccinated. We are very much in this direction, having administered close to 180 million doses in such a short time. These vaccines are found to induce higher neutralizing antibody titers than natural infection with very mild side effects in the fractional population to absolutely no side effects in the vast majority (Kyriakidis et al., 2021). Having said that, only time will reveal long-term side effects, if any, and the scientific community's integrity and transparency. The road ahead is also becoming razor-sharp as the SARS-CoV-2 is mutating rapidly, showing different variants in different countries, for which the vaccine should be tweaked appropriately. Nevertheless, we are confident that the scientists who have ferried us so far will also develop solutions for mutating virus.

But are vaccines always an answer? Should we not develop a good and natural immune system by leading a healthy lifestyle? Because with the next new virus, the world will run around again in frenzied chaos. As they say, good police (vaccine) can catch a thief, but if we have a good policing system (sound natural immune system in this context) in place, we could have ensured that theft did not happen in the first place. It is also about time to learn to not play with nature to prevent other zoonotic diseases which could be as infectious and deadly as SARS-CoV-2, if not more.

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